

**MASTISOPLEX® CRE-ART**  
**Frequently Asked Questions and Answers**

**What family members are detected for each of the target groups?**

The full list of family members detected by the **MASTISOPLEX®** CRE-ART kit is:

<b>Targets</b>	
<b>Family</b>	<b>Family members</b>
<b>OXA-48</b>	48, 162, 163, 181, 199, 204, 232, 244, 245, 247, 252, 370, 405, 416, 438, 439, 484, 505, 514, 515, 517, 519, 538, 546, 547, 566, 567, 731, 788, 793, 833
<b>OXA-23</b>	23, 27, 49, 73, 103, 133, 146, 165, 166, 167, 168, 169, 170, 171, 225, 239, 366, 398, 422, 423, 435, 440, 482, 483, 565, 657, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818
<b>OXA-24/40</b>	24, 25, 26, 40, 72, 139, 160, 207, 437, 653
<b>KPC</b>	2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46
<b>VIM</b>	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 67, 68
<b>NDM</b>	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 27, 28
<b>IMP</b>	1, 3, 4, 6, 10, 25, 26, 30, 34, 38, 40, 42, 52, 55, 61, 60

**Why is inhibition control DNA (ICDNA) added into the assay?**

The ICDNA is used to ensure there has been no inhibition or degradation of the sample during the assay set up. If a negative result is observed for the inhibition control (tube 8), this could be the result of DNA degradation, inhibition or failure to add ICDNA to the assay. Repeat testing should be carried out.

**What happens if a higher volume of sample reaction mixture is added to a CRE-ART pellet?**

We recommend that users follow the instructions for use. However, if an incorrect volume is added in error, there is the potential for a slower amplification which may cause a false negative result.

**What happens if a pellet isn't resuspended properly?**

As per the Instructions for Use, we recommend mixing during the reconstitution steps by gently pipetting up and down (see instructions for use). The pellets have been designed and manufactured to easily resuspend and it is unlikely that a pellet would not be fully resuspended. However, should this occur then re-mixing gently with a pipette should resolve the issue.

### **Does a specific 1.5ml tube have to be used?**

Any assay reaction tube may be used providing it is capped, compatible with the temperature and heating block of choice and can handle the volume required to set up the assay.

### **What happens if too much bacterial growth is incorporated into the reaction?**

Too much bacterial growth would cause an excess amount of DNA which could produce a high background and a false negative result due to the amplification reaching the maximum threshold of the instrument. The assay should be repeated with the correct amount of input DNA.

### **What happens if too little bacterial growth is incorporated into the reaction?**

Too little bacterial growth would cause an insufficient amount of DNA and the number of target copies present could fall below the limit of detection. It may not be possible to see an amplification profile in the 30 minute reaction time. See Instructions for Use for further information. The assay should be repeated with the correct amount of DNA if possible.

### **What kind of culture media can be used in order to be compatible with this assay?**

**MASTISOPLEX<sup>®</sup> CRE-ART** has been validated for use with Columbia Agar, Mueller-Hinton Agar, CHROMagar<sup>™</sup> mSuperCARBA<sup>™</sup>, KPC and ESβL chromogenic media. Any other media should be evaluated internally prior to diagnostic use. Please see the Instructions for Use for further information.

### **Will the inclusion of media 'agar jelly' inhibit the reagents in the assay?**

In-house tests have shown the presence of media 'agar jelly' from obtaining the colony does not inhibit amplification of the Inhibition Control DNA (ICDNA) or lead to the presence of false amplification in no template controls.

### **What happens if a sample is left in the heat block for too long?**

In house tests were conducted to determine if the length of incubation at 95°C had an effect on the Inhibition Control DNA (ICDNA). These tests showed there was no significant difference in the Ct value of results at incubation times of 5 minutes and 30 minutes 95°C.

### **What amplification detection equipment can I run the assay on?**

**MASTISOPLEX<sup>®</sup> CRE-ART** is compatible with any real-time detection equipment with a FAM detection channel. The assay has been validated on the ABI 7500, ABI 7500 FAST and the TS2 TubeScanner. Other thermal cyclers should be fully evaluated internally prior to diagnostic use.

### **When would a result be invalid?**

An invalid result would be observed when the inhibition control (tube 8) doesn't show a positive result profile. This would suggest there has been some inhibition and results have been compromised. A negative template control with a positive result in tube 1-7 would also be invalid, as would a negative result for a positive control. In these circumstances testing should be repeated. Checks should be made to ensure ICDNA is added to the assay and that all instrumentation is DNase free.

### **A curve isn't appearing until late in the reaction, why is this?**

This is likely to be a result of the DNA concentration. A lower DNA concentration will result in a longer time to amplify and as a result the amplification curve won't appear until later on in the reaction

### **The inhibition control is negative? What does this mean?**

This would indicate that there is inhibition of the sample and the testing should be repeated.

### **One of the result profiles is significantly lower than the other positives, but higher than the negatives – can this be reported as positive?**

Yes, this would indicate a positive result providing that the ICDNA is positive and shows no sample inhibition.

### **Can the Inhibition Control (ICDNA) be reused multiple times?**

Yes, the inhibition control can be reused for up to 10 samples. We would recommend that the reconstituted ICDNA is aliquoted into smaller volumes and stored at -20°C to avoid repeat freeze thawing.

### **Can a positive control be used with the assay?**

Yes. An inhouse positive control / ATCC strain for the target of interest can be used with **MASTISOPLEX<sup>®</sup> CRE-ART** if deemed appropriate. Alternatively, contact Mast Group Limited technical support for information about ordering a positive control.

### **How should the assay be stored?**

In order to achieve optimum results, we recommend that the assay is stored away from direct sunlight at 2-30°C. The internal control (ICDNA) should be stored at 2-8°C on the day of the test and at -20°C for long term storage. On the day of conducting tests, the re-constituted CRE-STRIP should be held at 2°C. to 8°C during assay set-up

**What are the limits of detection for each target?**

Target gene	Probit value Femtograms/ $\mu$ L (95% confidence interval)	Probit value DNA copy number/ $\mu$ L (95% confidence interval)
OXA-48	0.13fg/ $\mu$ L (0.07 to 0.75)	44 copies/ $\mu$ L (22.5 to 244.2)
OXA-23	0.28fg/ $\mu$ L (0.15 to 1.19)	92 copies/ $\mu$ L (49.7 to 386.7)
OXA-24/OXA-40	2.1fg/ $\mu$ L (1.05 to 2.02)	683 copies/ $\mu$ L (344 to 6576)
KPC	0.1fg/ $\mu$ L (0.05 to 0.58)	35 copies/ $\mu$ L (17.4 to 187.4)
VIM	0.1fg/ $\mu$ L (0.05 to 0.62)	35 copies/ $\mu$ L (16.6 to 195.7)
NDM	0.2fg/ $\mu$ L (0.08 to 1.36)	53 copies/ $\mu$ L (27.6 to 442.7)
IMP	2.7 g/ $\mu$ L (1.12 to 27.8)	879 copies/ $\mu$ L (364.8 to 9035.3)

Further information can be found in the Instructions for Use.

**Is it possible to know that the primers are working correctly?**

There are rigorous quality control checks during the manufacturing of **MASTISOPLEX<sup>®</sup> CRE-ART** that ensures optimal performance of all pellets, the correct location of each pellet and ensures there is no cross contamination. An in-house positive control could be used on the assay for internal quality control purposes if required.

**Is the test qualitative or quantitative?**

**MASTISOPLEX<sup>®</sup> CRE-ART** is a qualitative assay.